"Validation of Whole-Slide Imaging in the Primary Diagnosis of Liver Biopsies in a University Hospital"

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ABSTRACT

Background: Experience in the use of whole slide imaging (WSI) for primary diagnosis is limited and there are no comprehensive reports evaluating this technology in liver biopsy specimens.

Aims: To determine the accuracy of interpretation of WSI compared with conventional light microscopy (CLM) in the diagnosis of needle liver biopsies.

Methods: Two experienced liver pathologists blindly analyzed 176 consecutive biopsies from the Pathology Department at the Hospital Clinic of Barcelona. One of the observers performed the initial evaluation with CLM, and the second evaluation with WSI, whereas the second observer performed the first evaluation with WSI and the second with CLM. All slides were digitized in a Ventana iScan HT at 400x and evaluated with the Virtuoso viewer (Roche diagnostics). We used kappa statistics (κ) for two observations.

Results: Intra-observer agreement between WSI and CLM evaluations was almost perfect (96.6%, κ= 0.9; 95% confidence interval [95%CI]: 0.9-1 for observer 1, and 90.3%, κ= 0.9; 95%CI: 0.8-0.9 for observer 2). Both native and transplantation biopsies showed an almost perfect concordance in the diagnosis.

Conclusion: Diagnosis of needle liver biopsy specimens using WSI is accurate. This technology can reliably be introduced in routine diagnosis.

KEY WORDS: digital pathology, liver pathology, hepatic needle biopsies, intra-observer agreement

INTRODUCTION

Conventional light microscopy (CLM) has been the basic and, until recently, the only tool for the histological diagnosis of biopsy specimens. The development of the whole-slide Imaging (WSI) technology has started to change this picture in the last few years.

The basis of the WSI technology is the use of high throughput scanners able to create high quality digital reproductions of glass slides containing a complete histological section and WSI viewers that allow navigation across the virtual slide. These tools enable the use of the computer as a CLM. WSI has many practical applications that include education and teleconsultation [1–4]. In the last few years the medical community has shown increasing interest in the use of WSI for routine primary diagnosis [5–7].

Indeed, routine pathological diagnosis can benefit from the advantages of this technology. The WSI workstations are more ergonomic and facilitate a more efficient sign-out process. WSI allows viewing several slides at the same time on the same screen, which is particularly helpful for the evaluation of immuno- or histochemically stained slides that can be analyzed together with hematoxylin-eosin (H&E) staining (Figure 1). The digital viewers incorporate tools that enable making annotations, rotating the images and making precise measurements [8]. WSI has a much larger field of vision than CLM and a wider range of magnifications, including very low magnifications that are very useful for the evaluation of surgical specimens. WSI facilitates sharing images and information with clinicians and other pathologists. This is not only extremely useful in tumors boards, but also allows expert tele-consultation of difficult cases and frozen section intra-operative biopsies [9,10]. Finally, with WSI

algorithms can be used for the evaluation and quantification of immuhistochemical stains, resulting in a more objective evaluation [11–14]. This tool is likely to become essential to achieve standardized diagnoses in the near future.

Although WSI is considered to be comparable to CLM, adequate correlation between WSI and CLM diagnoses should be confirmed before this technology is used for primary diagnosis. The number of studies aimed at validating WSI in the routine diagnosis of the different areas of pathology is rapidly growing [15]. However, whereas relatively abundant information is available in some areas of pathology, validation studies are very scant or even absent in other areas, such as liver biopsy. Indeed, while a few studies have used this tool in research and automated image analysis [16–24], there is a complete absence of studies validating the use of WSI in needle liver biopsies, which may lead to reluctance in implementing this technology in routine diagnosis.

MATERIALS AND METHODS

Characteristics of the institution

This study was performed at the Department of Pathology in the Hospital Clinic (Barcelona, Spain). This department is composed of 16 pathologists, 8 residents and a variable number of fellows. The specimens are divided into 14 subspecialties, and the pathologists limit their practice to one or two areas. In 2015 the Department handled 43,678 specimens with 11,081 paraffin blocks. The number of liver needle biopsy specimens during this year was 230. The study was approved by the institutional ethics review board /HCB/2014/0514.

Sample size calculation

The highest rate of discrepancy between the original diagnosis by CLM and that by WSI was calculated to be 3%, with a non-inferiority margin for WSI review of 5%. A 1-sided binomial test was used for comparison at a level of significance of .05. The power to be achieved was 70%, and the level of significance was .05. Based on these assumptions, it was calculated that 100 cases would need to be reviewed to establish non-inferiority [25].

Specimens included in the study

All consecutive needle liver biopsy specimens received at the Department of Pathology of the Hospital Clinic in a 9-month period (February-October 2015) and assigned to the same expert pathologist were included in the study (n=176). This represented 76.5% of the total number of liver biopsies evaluated in 2015. All cases had a single paraffin block, containing one to five specimens (median 1). All specimens were routinely stained with H&E, Masson's trichrome and reticulin stain. Additionally, immunohistochemical stains were used for specific cases after the request of the pathologist. The total number of scanned slides was 1286. The biopsies included both native and transplanted livers (n=112 and n=64, respectively). The median age of the patients was 57 years (range 18-91).

Scanning process and characteristics of the WSI display

All the needle liver biopsies were scanned daily after CLM diagnosis. The scanning of the histological slides was performed on a Ventana iScan HT (Ventana Medical Systems, Tucson, AZ, USA) at a magnification of 400x. The scanning process run automatically, and includes the selection of the area that

contains the tissue, the determination of the focus points, the calibration, and the scanning. When more than one section are mounted on a single slide, the system scans all the sections. No specific quality control of the slides scanned was made prior to evaluation by the pathologist. The WSI produced are stored in a dedicated mass storage environment and linked to the pathology report, based on the recognition of a QR code on the slide label. Although WSI can be accessed through the pathology laboratory information system (LIS) software (Novopath, Vitrosoft SL, Sevilla, Spain), for the purposes of this study access to the WSI was made through the viewer.

The images were viewed with the Virtuoso viewer (Ventana), which works as a web browser and simulates a CLM. The images are shown using the same structure provided by the LIS. No specific software installation is required to visualize the WSI. The scanned images can be viewed up to a real magnification of 400x and up to 600x with a digital zoom, are always in focus, and have an optimized contrast and adjusted illumination. The viewer shows a thumbnail of the whole slide, which allows confirmation that all the material present on the glass slide has been included in the digital image and helps in the navigation through the slide. The WSI are displayed on a 30” Coronis fusion MDC4130 monitor which has a resolution of 4 Megapixels (Barco Electronic Systems, Barcelona, Spain).

CLM and WSI diagnosis

Two experts in liver pathology analyzed all cases. The first observer performed the initial evaluation with CLM, which was considered the reference for diagnostic attribution, and the second observation with WSI, whereas the second observer performed the initial evaluation with WSI and the second with

CLM. In order to avoid interference with the first diagnosis, the minimum washout period between the two observations was 1.5 months (range 1.5-4 months). All the histological slides of each case were scanned and evaluated. A summary with the basic original clinical information was provided to the pathologist in both evaluations in order to emulate the real clinical environment, and the pathologist could also request additional information. When performing the WSI, the pathologist was blinded to the original diagnostic report, as well as to the evaluation made by the other pathologist. In all cases, a main diagnosis was rendered in both evaluations. Additionally, in some specimens additional secondary diagnoses were also provided.

Concordance between CLM and WSI diagnosis and features evaluated

An independent pathologist not involved with the evaluation compared the original CLM and the WSI-based evaluations and judged the concordance of the two diagnoses. Concordance was classified as: a) complete agreement; b) minor discrepancy (slight differences which would not have any clinical or prognostic implications); and c) major discrepancy (differences with clinical and/or prognostic implications for the patient).

Some histological features were routinely evaluated in all the specimens: portal fibrosis (using a 0-4 scale), presence or absence of Mallory-Denk bodies, steatosis (using a 0-3 scale), and liver cell ballooning [26,27]. Portal inflammation, cholangitis and endothelitis were estimated using a 0-3 scale in all the acute rejection biopsies [28]. In the cases of cirrhosis and chronic hepatitis, necro-inflammatory activity (portal/periportal and lobular) was evaluated with a 0-3 scale [29–32]. Other characteristics were recorded when present.

Statistical analysis

The SPSS (SPSS IncTM140, Version 18, Chicago, IL, USA) was used for statistical analyses. The results for categorical variables are expressed as absolute numbers and percentages and 95% confidence intervals (95%CI). The Chi-squared or the Fisher's exact tests were used to compare the variables. The results were evaluated by unweighted Kappa statistics for two observations. This measure calculates the degree of agreement in classification over that which would be expected by chance and is scored as a number between 0 and 1. According to the Landis-defined categories the strength of agreement of the Kappa values (κ) is: 0 none, beyond chance; 0-0.20 slight; 0.21-0.40 fair; 0.41-0.60 moderate; 0.61-0.80 substantial; 0.81-1.00 almost perfect. For the kappa value calculation, the main diagnoses were grouped into nine categories for the native livers and six categories for the transplanted livers. The diagnostic categories for the native livers included: a) slight changes (including isolated steatosis), b) venous congestion, c) autoimmune diseases (autoimmune hepatitis and primary biliary cirrhosis), d) steatohepatitis, e) acute hepatitis, f) chronic viral hepatitis g) cirrhosis, h) malignant tumors (primary or metastatic), and i) other diseases. The diagnostic categories for the transplanted livers included: a) slight changes; b) autoimmune hepatitis, c) steatohepatitis, d) hepatitis C virus infection, e) acute cellular rejection, and f) chronic rejection.

RESULTS

Intra-observer and inter-observer agreement

The overall intra-observer agreement between the CLM and the WSI diagnoses was 96.6% (κ= 0.9; 95% CI: 0.9-1) for the observer 1 and 90.3% (κ= 0.9; 95% CI: 0.8-0.9) for the observer 2. There were four minor discrepancies between the CLM and the WSI diagnoses for observer 1 and 14 for observer 2. None of the discrepancies were related to a poor quality of the WSI image or to insufficient magnification. The diagnoses of carcinoma showed 100% concordance in all four evaluations. The overall inter-observer agreement between the CLM diagnoses performed by observer 1 and 2 was 92.6% (κ= 0.9; 95% CI: 0.9-1) and 96.6% (κ= 1; 95% CI: 0.9-1) for the WSI diagnoses.

The intra-observer agreement between CLM and WSI for the native liver biopsies (n= 112) was 95.5% (κ= 0.9; 95% CI: 0.9-1) for observer 1 and 90.2% (κ= 0.9; 95% CI: 0.8-0.9) for observer 2. The inter-observer agreement between the CLM diagnoses performed by observer 1 and 2 was in this group of native liver biopsies was 92.9% (κ= 0.9; 95% CI: 0.9-1) and 87.5% (κ= 0.9; 95% CI: 0.8-0.9) for the WSI diagnoses. Table 1 shows the intra-observer (WSI vs. CLM) for the two observers and the inter-observer agreement for CLM and WSI for each specific diagnostic group for the 112 native liver specimens.

Table 2 shows the intra-observer (WSI vs. CLM) for the two observers and the inter-observer agreement for CLM and WSI for each specific diagnostic group for the 64 transplantation biopsies. The intra-observer agreement between CLM and WSI for the transplanted liver biopsies was 93.7% (κ= 0.9; 95% CI: 0.8-1) for observer 1 and 87.5% (κ= 0.8; 95% CI: 0.7-0.9) for observer 2. The inter-observer agreement between the CLM diagnoses performed by observer 1 and 2 was in this group of transplanted liver biopsies was 89.1% (κ= 0.8; 95% CI: 0.7-1) and 87.5% (κ= 0.8; 95% CI: 0.7-0.9) for the WSI diagnoses.

Agreement between WSI and CLM in relevant liver changes

Table 3 shows the intra-observer (WSI vs. CLM) and the inter-observer (WSI vs. WSI and CLM vs. CLM) agreement in the evaluation of relevant histological features in the native livers (steatosis, liver cell ballooning, presence or absence or Mallory-Denk bodies, portal/peri-portal inflammatory activity and necrosis and lobular necrosis and inflammatory activity). Table 4 shows the intra-observer (WSI vs. CLM) and the inter-observer (WSI vs. WSI and CLM vs. CLM) agreement in the evaluation of major histological features in transplanted livers (portal inflammation, cholangitis and endothelitis).

DISCUSSION

This is the first study evaluating the accuracy of WSI diagnosis in the routine practice of needle liver biopsies. Our results show a high intra-observer concordance between the CLM and the WSI evaluations (over 90% for both obseervers) in the diagnoses of a large series of routine needle liver specimens. The kappa value, considered as a measure of the level of intra- and inter-observer agreement corrected by chance, was almost perfect (0.9 for both observers) and 0.9-1 for the inter-oberver comparisons of the CLM and the WSI evaluations. The percentage of discrepancies between the CLM and WSI diagnoses observed in our study was below 10%, and only minor discrepancies were identified. Neither had an impact on patient management. More importantly, none of the discrepancies was related to a poor quality of the WSI image or to insufficient magnification. All the discrepancies observed were associated with either the small size of the material or to the intrinsic difficulty of

the case. Thus, our results confirm that WSI may confidently be used for primary histological diagnosis of liver biopsies.

A number of studies have shown that there is a substantial variation between and within observers in the evaluation of liver biopsy specimens. These studies are limited to specific diseases such as non-alcoholic steatohepatitis and chronic viral hepatitis [33–37]. In an intra-observer concordance study including 50 biopsies oriented as non-alcoholic steatohepatitis Kleiner et al reported a kappa value of 0.61 [34]. Our study showed a higher rate of concordance in the evaluation of steatohepatitis with a kappa value ranging from 0.7 to 0.9 in the different comparisons, although the number of cases with this diagnosis was much lower and included both alcoholic and non-alcoholic steatohepatitis. Three studies have evaluated intra-observer concordance in the diagnosis of chronic viral hepatitis. The evaluation of fibrosis grade and stage in these studies showed kappa values ranging from 0.72 and 1 [35–37], which were comparable with the concordance rates observed in our study (0.7 to 0.9). These discrepancies have mainly been attributed to the inherent intra-observer variability in the diagnosis of needle liver biopsy specimens. Interestingly, some of these studies analyzed a number of histological features separately, showing high concordance rates for steatosis ($\kappa = 0.79$), periportal necrosis ($\kappa = 0.74$) and fibrosis ($\kappa = 0.86$) and lower values for lobular necrosis ($\kappa= 042$) [33,34]. In the present study, the concordance observed for all these histological findings showed even better results. A possible limitation of our study is the lower number of cases of each particular disease included in the analysis compared to previous reports [33–37].

However, our study was designed to evaluate the reliability of the WSI tool for the diagnosis of any liver lesion and not specifically for a single disease.

Interestingly, the specific analysis of liver transplantation specimens (n=64) showed a high intra-observer concordance that remained almost perfect (93.7%; κ= 0.9 for observer 1, 87.5, κ= 0.8 for observer 2). There were no differences in the diagnosis of rejection.

The results obtained in our study with the liver biopsies are comparable to other validation studies conducted in other areas of pathology, such as breast [38], skin [39], gastrointestinal [40,41], prostate [42–46], gynecological [25], renal [46,47] or pediatric pathology [48,49] which show similar high rates of concordance between CLM and WSI diagnoses. Thus, the results of all these studies indicate that WSI should be considered as a validated tool, almost equivalent to the CLM. In keeping with this assumption, the guidelines and recommendations of the College of American Pathologists, the Canadian Association of Pathologists and the American Telemedicine Association for adequate validation of WSI before its use in routine diagnosis do not require a validation for each specific area [1,4]. These recommendations indicate that only 60 samples per pathologist should be evaluated in order to ensure the familiarity of the pathologist with the new tool. Indeed, as with any other tool, there is a learning curve for WSI [25,50–54].

Remarkably, the pathologist did not report any difficulty in rendering the diagnosis at the magnification used in this study (400x). A 200x magnification is considered as appropriate to achieve a correct diagnosis in most previously published studies evaluating other areas of pathology [25,42,48,50,55–57]. However, this scanning magnification may not be sufficient for some areas,

such as the liver due to the small size of the specimens and the need to evaluate subtle changes that frequently require the use of high magnification.

The introduction of the WSI technology may significantly improve the diagnosis of routine needle liver biopsy specimens taking into account the advantage of the possibility of viewing multiple slides at the same time with this technique. Indeed, this advantage can be very useful in liver pathology since several stains are often used and WSI facilitates tele-consultation. Finally, the future development of computer-assisted diagnostic algorithms is likely to help reduce intra- and inter-observer variability. However, many issues should be addressed to make this implementation feasible and cost-efficient, such as the cost of the scanners [4,8,58–61], the costs associated with the maintenance of the system and the storage of the images and legal issues related to the use of WSI for primary diagnosis, including image storage and patient confidentiality. Approval is currently being sought from the US Food and Drug Administration (FDA) for the use of WSI in primary diagnosis. In the meantime, WSI is being increasingly used in several centers around the world.

In conclusion, the diagnosis of needle liver biopsies using WSI has high intra-observer concordance with the results of CLM evaluation. Our results confirm that WSI can be safely used for primary histological diagnosis of liver biopsies, including native and transplantation specimens.

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FIGURE LEGEND

Figure 1. The WSI viewer may simultaneously show and synchronously move several slides of a case. This is particularly helpful in the evaluation of liver biopsy specimens since it allows the analysis of an H&E stained slide together with histochemically and/or immunohistochemically stained slides.

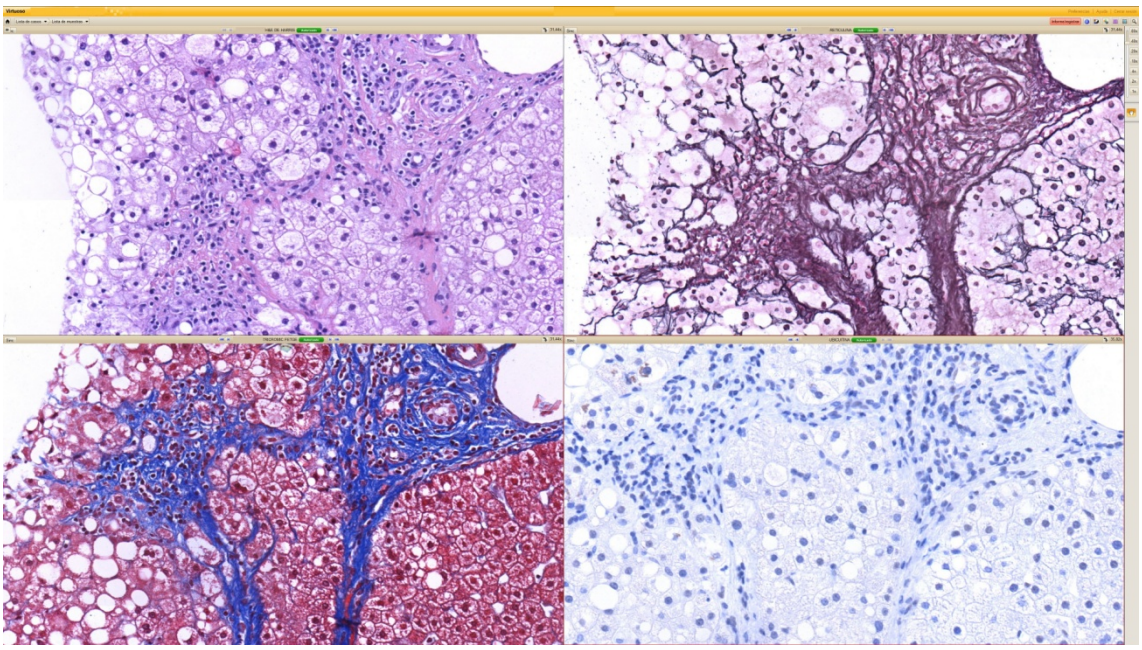

Table 1: Intra-observer (whole slide imaging [WSI] vs. conventional light microscopy [CLM]) for the two observers and Inter-observer agreement for CLM and WSI in the diagnosis of native liver specimens (n=112).

Diagnosis	n	Intra-observer agreement Observer 1	Kappa value (95% CI)	Intra-observer agreement Observer 2	Kappa value (95% CI)	Inter-observer agreement CLM	Kappa value (95% CI)	Inter-observer agreement WSI	Kappa value (95% CI)
Mild changes *	22	98.3	0.9 (0.9-1)	94.9	0.9 (0.8-0.9)	96.0	0.9 (0.8-1)	96.0	0.9 (0.8-1)
Venous congestion	4	100	1 (NA)	98.9	0.9 (0.5-1)	98.3	0.7 (0.4-1)	99.4	0.9 (0.7-1)
Autoimmune diseases #	15	99.4	1 (0.9-1.0)	98.3	0.9 (0.8-1)	98.9	0.9 (0.8-1)	97.7	0.9 (0.7-1)
Steatohepatitis	12	98.3	0.9 (0.8-1)	97.7	0.8 (0.7-1)	97.7	0.8 (0.7-1)	96.0	0.7 (0.6-0.9)
Acute hepatitis &	7	99.4	0.9 (0.8-1)	98.3	0.8 (0.6-1)	99.4	0.9 (0.8-1)	98.3	0.8 (0.6-1)
Chronic hepatitis **	14	97.7	0.9 (0.9-1)	94.9	0.8 (0.7-0.9)	97.7	0.9 (0.9-1)	93.7	0.8 (0.7-0.9)
Cirrhosis	19	100	1 (NA)	100	1 (NA)	98.9	0.9 (0.8-1)	98.9	0.9 (0.8-1)
Tumors ##	14	100	1 (NA)	100	1 (NA)	100	1 (NA)	100	1 (NA)
Other &&	5	100	1 (NA)	98.9	0.8 (0.6-1)	99.4	0.9 (0.8-1)	99.4	0.9 (0.8-1)

95%CI: 95% confidence interval; * includes mild to moderate macrovesicular steatosis (n=15) and mild non-specific changes (n=7); # includes autoimmune hepatitis (7 cases) and primary biliary cirrhosis (8 cases); & includes four toxic acute hepatitis, two acute B hepatitis and one hepatitis of unknown origin; ** includes 10 chronic hepatitis C, two chronic hepatits B and two drug induced hepatitis; ## includes one cholangiocarcinoma, six metastatic carcinomas, six hepatocellular carcinomas and one hemangioma; && includes one case each of schistosomiasis, cystic fibrosis, graft versus host disease, sclerosing cholangitis and nodular regenerative hyperplasia. NA: not applicable

Table 2: Intra-observer (whole slide imaging [WSI] vs. conventional light microscopy [CLM]) for the two observers and Inter-observer agreement for CLM and WSI in the diagnosis of liver transplantation biopsies (n=64).

Diagnosis	n	Intra-observer agreement Observer 1	Kappa value (95% CI)	Intra-observer agreement Observer 2	Kappa value (95% CI)	Inter-observer agreement CLM	Kappa value (95% CI)	Inter-observer agreement WSI	Kappa value (95% CI)
Mild changes	23	95.3	0.9 (0.8-1)	92.2	0.8 (0.7-1)	93.7	0.9 (0.7-1)	90.6	0.8 (0.6-1)
Autoimmune hepatitis	2	100	1 (NA)	100	1 (NA)	100	1 (NA)	100	1 (NA)
Steatohepatitis	1	100	1 (NA)	100	1 (NA)	98.4	7 (0.0-1)	98.4	0.7 (0.0-1)
Chronic hepatitis *	20	100	1 (NA)	100	1 (NA)	100	1 (NA)	100	1 (NA)
Acute cellular rejection	15	95.3	0.9 (0.7-1)	89.1	0.7 (0.5-0.9)	90.6	0.7 (0.6-0.9)	93.7	0.8 (0.7-1)
Chronic rejection	1	100	1 (NA)	100	1 (NA)	100	1 (NA)	100	1 (NA)
Other lesions #	2	100	1 (NA)	100	1 (NA)	100	1 (NA)	100	1 (NA)

95%CI: 95% confidence interval; NA: not applicable; * hepatitis C virus reinfection; # Includes one case of preservation injury and one insufficient biopsy

Table 3: Intra-observer (whole slide imaging [WSI] vs. conventional light microscopy [CLM]) for the two observers and Inter-observer agreement for CLM and WSI in the evaluation of major histological changes in the native livers (n=112).

Histological feature	Intra-observer agreement Observer 1	Kappa value (95% CI)	Intra-observer agreement Observer 2	Kappa value (95% CI)	Inter-observer agreement CLM	Kappa value (95% CI)	Inter-observer agreement WSI	Kappa value (95% CI)
Fibrosis *	90.9	0.8 (0.8-0.9)	92.6	0.9 (0.8-0.9)	82.9	0.7 (0.6-0.9)	90.3	0.8 (0.8-0.9)
Steatosis #	91.1	0.9 (0.8-0.9)	99.1	1 (NA)	92.9	0.9 (0.8-1)	92.0	0.9 (0.8-0.9)
Liver cell ballooning &	98.2	0.9 (0.9-1)	100	1 (NA)	96.4	0.9 (0.8-1)	98.2	0.9 (0.9-1)
Mallory-Denk bodies &	98.2	0.9 (0.8-1)	99.5	1.0 (0.9-1.0)	97.3	0.9 (0.7-1)	100	1 (NA)
Portal/peri-portal inflammatory activity and necrosis #,**	92.9	0.8 (0.6-0.9)	92.0	0.8 (0.7-0.9)	78.6	0.5 (0.3-0.6)	84.8	0.6 (0.5-0.8)
Lobular necrosis and inflammatory activity #,**	96.4	0.9 (0.8-1)	93.7	0.9 (0.7-1.0)	87.5	0.7 (0.5-0.8)	86.6	0.7 (0.5-0.8)

95%CI: 95% confidence interval; * Graded on a 0-4 scale; # Graded on a 0-3 scale; # Evaluated as absent or present; ** Portal/peri-portal inflammatory activity and necrosis and lobular necrosis and inflammatory activity were evaluated only in the cases with a diagnosis of cirrhosis (n=19) and chronic hepatitis (14 in native livers and 20 in transplanted livers)

Table 4: Intra-observer (whole slide imaging [WSI] vs. conventional light microscopy [CLM]) for the two observers and Inter-observer agreement for CLM and WSI in the evaluation of the main histological features in the transplanted livers (n=64).

Histological feature	Intra-observer agreement Observer 1	Kappa value (95% CI)	Intra-observer agreement Observer 2	Kappa value (95% CI)	Inter-observer agreement CLM	Kappa value (95% CI)	Inter-observer agreement WSI	Kappa value (95% CI)
Portal inflammation	100	1 (NA)	90.6	0.8 (0.6-0.9)	85.9	0.7 (0.5-0.9)	95.3	0.9 (0.8-1)
Cholangitis	96.9	0.9 (0.8-1)	98.4	1 (0.9-1)	95.3	0.9 (0.7-1)	92.2	0.8 (0.6-1)
Endothelitis	96.9	0.9 (0.8-1)	93.7	0.8 (0.8-1)	93.7	0.8 (0.7-1)	95.3	0.9 (0.7-1)

95%CI: 95% confidence interval; All features were graded on a 0-3 scale.